I. Remarks

Claims 31-59, 61 and 62 are pending in the application. Claims 31-42 and 46-59 were withdrawn as directed to a non-elected invention. Applicants reserve the right to pursue these withdrawn claims in this or a related application.

Claims 43-45, 61, and 62 are under examination. Claims 43-45, 61, and 62 were rejected under 35 U.S.C. § 112, first paragraph for lack of enablement and written description. Claims 43-45, 61, and 62 were rejected under 35 U.S.C. § 112, second paragraph. Claims 43-45, 61, and 62 were rejected under 35 U.S.C. § 102 (b).

Each of the rejections raised in the Office Action is addressed below.

II. Amendments

In claims 43, 44, and 61, the term "pluripotent" has been omitted.

Claims 43 and 44 have been amended to include the tissues from which the cells of the invention originate. Support for this amendment can be found in the specification, for example, in Example 1, or on page 12, lines 1-4:

> *A first object of the present invention is the identification and characterization of skeletal precursor cells in a wide range of easily accessible and expandable sources. Easily accessible tissues include among others, periosteum, bone marrow and synovial membrane.*

Moreover, the isolation of the claimed cell population from these tissues is illustrated in the Examples section of the specification.

In claim 62, the phrase "or a marker or factor co-expressed or co-detectable with said FGFR3" has been replaced with *"or another marker of the mature chondrocyte phenotype"*. Support for this language is found in the specification, for example, on page 31, lines 9-11:

> *The mature chond[r]ocyte phenotype is heralded by the appearance of type II collagen, type X collagen, FGFR3 (fibroblast growth factor 3) and BMP2.*

New dependent claim 63 finds support in the specification, at least in the last sentence of the summary of the invention, which states:

> *A fifth object of the present invention is the co-implantation of expanded skeletal precursor cells and chondrocytes for in vivo cartilage repair.*

This aspect of the invention is further supported by the specification on page 22, line 22 to page 23, line 1.

New dependent claim 64 corresponds to the features of claim 61, and is supported by the specification, for example, on page 17, lines 10-11:

> *Particularly, the present invention includes cells having the presence of an negative marker such as FGFR3 combined with the presence of a positive marker*

and the section on page 31, lines 9-11 cited above:

> *The mature chond[r]ocyte phenotype is heralded by the appearance of type II collagen, type X collagen, FGFR3 (fibroblast growth factor 3) and BMP2.*

III. Rejections under 35 U.S.C. § 112, First Paragraph – Enablement

Claims 43-45, 61, and 62 were rejected under 35 U.S.C. § 112, first paragraph as

failing to comply with the enablement requirement. For the following reasons, this rejection should be withdrawn.

The claims, as amended, are directed to a culture of viable, differentiated, precursor cells retaining the intrinsic potential of multilineage differentiation, isolated from periosteum, bone marrow, or synovial membrane and expanded, that have entered a post-natal skeletal differentiation pathway leading to skeletal or connective tissue, wherein the cells express a positive embryonic marker which is CDMP-1 or a marker co-expressed and/or co-detectable with CDMP-1.

It was asserted in the Office Action that a skilled person could not predictably arrive at the claimed cells, because the skilled person would not know how to isolate the cells only by using a marker that is expressed postnatally in various tissues, including brain and placenta.

Without acquiescence to this assertion, the claims have been amended to explicitly recite the source of the cells. Accordingly, in view of this amendment and given Applicants' demonstration that within these tissues, only skeletal precursor cells were found to be CDMP-1 positive, it is submitted that the specification provides sufficient guidance to the skilled person to isolate the presently claimed cells.

The Office has argued that the dependent claims specifying the negative marker "are not within the scope of the claims" and that the claims are not enabled over their full scope. Applicants disagree.

First, Applicants note that cells characterized by additional markers are encompassed within the scope of independent claims 43 and 44. Indeed, in addition to the expression of a positive marker, the cells of the invention can be characterized by the absence of a negative marker, such as FGFR-3. The Office has expressed doubts on how the absence of a negative marker can characterize the cells. However, as detailed in the specification, the absence of expression of certain proteins can be of similar importance as the positive expression of other proteins. As is detailed in the specification, the mature chondrocyte phenotype is heralded by the appearance of certain markers including type II collagen, type X collagen, FGFR3, and BMP2. Accordingly, the absence of these markers further characterizes a precursor cell.

The Office has further questioned how markers can be co-expressed with a negative marker. Applicants respectfully submit that, similar to co-expression, absence of expression can be compared between markers at different time points, whether positive or negative markers. Indeed, it is clear that a marker which is not expressed by the cells in the same way as FGFR-3, for example, a marker the expression of which is, similar to FGFR-3, linked to the chondrocyte phenotype and accordingly is absent during the precursor stage of cells, is a marker that it "co-expressed" with the negative marker FGFR-3. Accordingly, such negative markers can be identified when comparing the expression of mature cells and precursor cells.

Nonetheless, in order to further the allowance of the present application, Applicants have limited the claim to refer to "FGFR-3 or another marker of the mature chondrocyte phenotype".

The Office has argued that the amount of experimentation required to make and use the full scope of the claimed invention is more than simply routine, because CDMP-1 is expressed in various tissues and one of skill in the art could not use this marker to uniquely identify the claimed cell populations. As a consequence, one of skill would need to practice undue experimentation to first identify unknown markers and then determine whether the markers were expressed solely in the claimed cell types in order to uniquely identify the claimed cell population.

Applicants submit that the claims, as presently amended, do not require any undue experimentation. Applicants have demonstrated the presence of CDMP-1 as a reliable marker for skeletal precursor cells in these tissues (Example 3). Furthermore, methods for isolating cell populations based on a marker profile are known to the skilled person and would not require undue experimentation. Accordingly, this basis of the enablement rejection should be withdrawn.

Regarding the rejection for the purposes of therapeutic benefit, the Office has based this rejection on two arguments. The first argument relates to what has been discussed above, i.e. the ability to isolate reliably a cell population based on the expression of CDMP-1. As indicated, this aspect is rendered moot by the amendments of

the claims presented herewith.

In the second argument, the Office has reiterated that the working examples fail to correlate to a therapeutic result, as they are directed to injection of immunodeficient, nude mice which would not be considered a model for an immunocompetent individual, and that implantation of stem cells to provide therapy is found to be unpredictable.

Applicants maintain that this rejection is inappropriate. The specification teaches the formation of cartilage *in vitro* using the cells of the invention (Example 6), as well as the enhancement of the cartilage forming ability of chondrocytes with the cells of the invention, both *in vitro* and *in vivo*. Thus, Applicants' data provide objective enablement, which is all that is required under §112, first paragraph.

In addition, Applicants submit that the Office's reliance on Hui et al. to support its enablement rejection is misplaced. Hui et al. do not show that it would not be predictable to isolate a particular cell type with markers. Instead, Hui et al. proclaim that:

> *Future research should be directed at better characterisation of this cell population, including identifying unique markers and mapping lineage development.* (page 211, conclusion).

This is exactly what Applicants have done. Furthermore, Hui et al. state that:

> *Current research on human adult stem cells indicates significant potential for use in the development and regeneration of tissues, particularly in the field of transplantation. Due to the minute possibility of such cells being rejected by the patient, it would be extremely advantageous to isolate cells from the patient, direct the specialisation of the cells and transplant them back into the patient. Now, MSCs are being expected to treat a variety of clinical conditions, including large segmental defects, bone fractures or wounds that have severe scarring, infections, avascular tissue with a poor blood supply, non-union situations where bones are not fully joined and to*

treat the effects of irradiation and chemotherapy. (page 206, paragraph bridging column 1 and 2; emphasis added).

Moreover, Applicants emphasize that the cells of the present invention are characterized as multipotent precursor cells which have entered a post-natal skeletal differentiation pathway. Such cells are not 'unpredictable' as effectively demonstrated in Applicants' *in vivo* Examples found in the specification.

In addition, as applied to the amended claims, reliance on Hui et al. is misplaced because the claims now refer to a specific cell type for use in the engineering of tissues. Accordingly, the observation by Hui et al. that a further characterization of the cells would allow their use in the treatment of varying conditions, supports the presently claimed invention. Indeed, by characterizing the cells as skeletal precursor cells, no "undue experimentation" is required as this cell population has been demonstrated to generate cartilage *in vivo*.

The Office has further indicated that a nude mouse model is inappropriate as a model for an immunocompetent organism. Applicants disagree. Indeed, it should be realized that, in practice, the treatment of humans according to the present invention will generally involve autologous cells, whereby the issue of immunocompetence is less relevant. Indeed the immunocompetence of the cells used in cellular therapy is an issue independent of the concept of the present invention. This is also supported by Hui et al. where it is stated that autologous cell transplantation results in only a 'minute possibility of such cells being rejected by the patient'. Moreover, alternative methods exist for

reducing the immunogenicity of heterologous cells.

The main issue, however, in the context of the present invention, is whether the cells of the invention are capable of producing stable hyaline cartilage upon isolation and injection into a mammal. Indeed, one of the biggest drawbacks of autologous chondrocyte transplantation is the loss of cartilage-forming ability of chondrocytes resulting from *in vitro* expansion. This aspect of the invention is very accurately demonstrated in the nude mouse model. Indeed, by using nude mice, it is possible to work with heterologous cells. This allows the isolation of a higher number of cells (from another organism) which is comparable to the situation in humans. It is noted that the mechanisms of cartilage formation in nude mice is similar to that of wild-type mice and accordingly the evaluation of cartilage production by a cell population is routinely measured in nude mice. As taught by Applicants (see, for example, Example 7), co-implantation of freshly isolated chondrocytes with skeletal precursor cells is able to substantially reduce the number of chondrocytes needed for successful joint surface defect repair. Such co-implantation also results in a remarkable enhancement of the amount of cartilage produced.

Although acknowledging increased cartilage formation, the Office has asserted that such increased cartilage formation does not provide a nexus to integration and function of the claimed cells to repair damaged or malfunctioning tissue. Applicants strongly disagree. Indeed, autologous chondrocyte transplantation is a widely accepted

technique for repair of joint surface defects (see page 10 of the specification, lines 10-12). This procedure has been demonstrated to effectively result in repair. The main limitation of this technique, prior to the present invention, has been the required number of cells for full repair. Given the fact that an increased number of chondrocytes is formed using the methods of the invention, the Office has failed to provide substantive reasons why implantation of the cell population of the present invention, would fail to have therapeutic benefit.

For all the reasons above, it is submitted that the specification fully enables the practice of the claimed invention and it is respectfully requested that the enablement rejection be withdrawn.

IV. Rejections under 35 U.S.C. § 112, First Paragraph – Written Description

The Office has indicated that the claims require essential or critical elements not adequately described in the specification. Examples include the presence of CDMP-1 in brain, where one would not expect the cells of the invention to be present, and lack of guidance to identify markers co-expressed or co-detectable with other markers.

Applicants submit that this rejection is rendered moot in view of the present amendment to the claims, specifying the origin of the cells.

As for the markers that are co-expressed with CDMP-1, Applicants point to the definition of co-expression provided in the specification, for example, on page 13, lines

17-31. It is clear that co-expression is assessed in the same cells as expression of CDMP-1, so these cells do not form a separate 'genus'. The nature of the marker is not important, but can be "a recognizable cell surface marker, detectable via polyclonal or monoclonal antibodies and/or specific ligands" (page 13, lines 28-30). Following the guidance in the specification, specifically the RT-PCR procedure described in Example 3, it is possible, using routine experimentation, to find other markers.

With regard to the negative markers, Applicants submit that the rejection is also rendered moot in view of the present amendment to the claims.

The Office has also asserted that the specification does not provide guidance on identifying precursor cells that have entered a postnatal differentiation pathway leading to skeletal or connective tissue, even using CDMP-1. This is respectfully contradicted, as it is precisely the expression of CDMP-1 that characterizes these cells. This is stated repeatedly throughout the specification, for instance on page 17, lines 16-22:

> *Evidence is provided that the expression of CDMP-1 qualifies a certain culture expanded cell population as skeletal precursor cells. This is an unexpected result, since CDMP-1 has always been known to promote chondrogenic differentiation and never linked to the phenotype of skeletal precursor cells. Regardless the source, cells are culture expanded and assessed by RT-PCR analysis for the expression of CDMP-1. Only the CDMP-1 expressing cells can be successfully processed to be directed into a specific differentiation pathway of any skeletal connective tissue, including cartilage.*

And, in Example 3, page 24, lines 24-29:

> *CDMP-1 was found expressed only by skeletal precursor cell populations at all the passages examined up to 18, and not by other cell populations. We indeed demonstrated that only the CDMP-1 marked culture expanded cells*

under specific conditions can differentiate e.g. towards chondrogenesis, while the CDMP-1 negative cells under the same conditions are not capable of undertaking any skeletal differentiation pathway.

Accordingly, Applicants submit that, by the very identification of the CDMP-1 marker have provided guidance on how one would discern between cells that have entered a postnatal differentiation pathway. Therefore, Applicants assert that more than adequate guidance is offered in the written description to allow one skilled in the art to isolate the cell population instantly claimed.

In short, one of skill in the art would, at the time of filing, understand what is encompassed by the language of the amended claims and, accordingly, would recognize that Applicants were in possession of the invention as now claimed. The written description requirement, with regard to such language, has been met.

V. Rejections under 35 U.S.C. § 112, Second Paragraph

The Office has objected to the use of the term 'pluripotent' in connection with the cells of the invention. In view of the present amendment, this rejection should be withdrawn.

VI. Rejections under 35 U.S.C. § 102(b)

Claims 43-44, 61 and 62 were rejected under 35 U.S.C. § 102(b) as anticipated by Takahashi et al. (J. Clin. Invest., 83: 543-550 (1989)).

Applicants submit that this rejection is rendered moot in view of the present amendment. Indeed, Takahashi et al. do not describe an isolated population of CDMP-1 positive cells. Furthermore, Takahashi et al. do not describe the use of this population in a therapeutic composition for the treatment of cartilage defects.

Claims 43-45, 61 and 62 were rejected under 35 U.S.C. § 102(b) as anticipated by Erlacher et al. (Arthritis & Rheumatism, 41(2): 263-273 (1998)).

Again, in view of the present amendment to the claims, it is submitted that this rejection is inappropriate. Erlacher et al. identify CDMP-1 expressing cells in cartilage. Erlacher et al. do not describe cells from bone marrow, periostium or synovial fluid. Erlacher et al. have not isolated these cells, nor defined their characteristics. Accordingly, Erlacher does not describe the cell cultures or the therapeutic compositions presently claimed.

Claims 43-45, 61, and 62 were also rejected as anticipated by Chang et al. (JBC, 269(45): 28227-28234 (1994)).

Chang et al. demonstrate that CDMP-1 post-natally can only be detected in articular and cricoid cartilage (page 28233, 1st column, lines 10-13). Chang et al. do not disclose that cells expressing CDMP-1 are isolated from bone marrow, periosteum or the synovial membrane.

Chang et al. have used cells from cartilage, part of which expressed CDMP-1, as an implant in immunocompetent rats. Again, Chang et al. doe not disclose a therapeutic

composition comprising a precursor cell population expressing CDMP-1 obtained from bone marrow, periosteum or the synovial membrane.

Claims 43-45, 61, and 62 were rejected as anticipated by Kyoizumi et al. (Blood, 79(9): 1704-1711 (1992)). Kyoizumi et al. describe the transplantation of fragments of human fetal femurs and tibia into SCID mice (see Abstract, first sentence; page 1704, 2nd column, SCID-hu mice paragraph, last two sentences; page 1705, 2nd column, line 1-5). No mention is made of isolation of cells or their expansion, let alone cells having the specific characteristics of the present invention; they transplanted tissue fragments. Indeed, in Kyoizumi et al. the tissues used for transplantation are described to be extracted, placed in medium, shipped and transplanted into SCID mice within 36 hours (page 1704, 2nd column, lines 8-15 of the SCID-hu mice paragraph).

None of the aforementioned references teach or suggest the presently claimed invention, as each does not describe the isolation of a population of CDMP-1 expressing cells. The section 102 rejection should therefore be withdrawn.

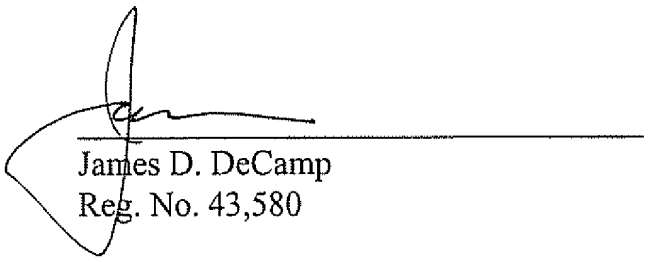
CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 27-Sep-2006



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